



# Diversity and Dynamics of the Canadian Coastal *Vibrio* Community: an Emerging Trend Detected in the Temperate Regions

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**ABSTRACT** *Vibrio* species are indigenous to the marine and estuarine environments around the world and are the leading cause of water- and seafood-borne illnesses due to conditions favoring the transmission and growth of the species. Horizontal gene transfer, recombination, and mutation enable *Vibrio* spp. to adapt rapidly to environmental challenges from biotic and abiotic parameters, including temperature, salinity, and nutrient status of the coastal waters. This surveillance study provides evidence of *Vibrio cholerae* emerging in the temperate estuaries of Canada, thereby redefining the diversity and dynamics of its coastal *Vibrio* population. The presence of the pathogenic context in *Vibrio parahaemolyticus* was also detected with an increasing trend during the study period.

**IMPORTANCE** Proliferation and abundance of the harmful biotypes of *Vibrio* spp. in the estuaries of Canada indicate the possibility of producing contaminated seafood for human consumption. The findings of this surveillance study may lead to awareness which may help efforts to reduce the occurrence of illnesses or outbreaks caused by *Vibrio* spp. in seafood.

**KEYWORDS** Canada, seafood safety, *Vibrio* species, surveillance

**V**ibrios are ubiquitous in marine and estuarine environments around the world, with more than 100 species identified, among which the most clinically significant species are *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* (1, 2). Cholera, caused by *V. cholerae*, is known to be linked to specific seasons and biogeographical zones based on warmer temperatures and ecology; it is an ancient disease which has killed millions of people from the warm tropical to the cold temperate regions of the world (1–4). This disease had disappeared from most of the developed world by the end of the 20th century, but it remained endemic in many tropical regions (4). In recent years, cholera has been detected in and reported from many developed countries in the temperate regions of the world (5–9). Among the prominent halophilic *Vibrio* spp., *V. parahaemolyticus* is the leading cause of seafood-associated illness around the world and, in North America, it prevails with extreme diversity (6, 8). Since emerging in the Far East (Japan) in the 1950s, this species has caused many seafood-borne outbreaks in the world by the identified pandemic strain and its serovariants (3, 10). The majority of the clinical isolates carry one or both of two hemolysin genes, encoding thermostable direct hemolysin (TDH) (11) and TDH-related hemolysin (TRH) (12). *V. vulnificus* is a highly lethal and opportunistic pathogen known to cause most seafood-related mortality in the United States (13). This surveillance study highlights two major trends of concern to human health in Canada as well as other temperate regions of the world, (i) the emergence of *V. cholerae* and (ii) the increase in detection of potentially pathogenic *V. parahaemolyticus* strains, isolated from molluscan shellfish harvested in Canada during the warmer months of 2006 to 2016.

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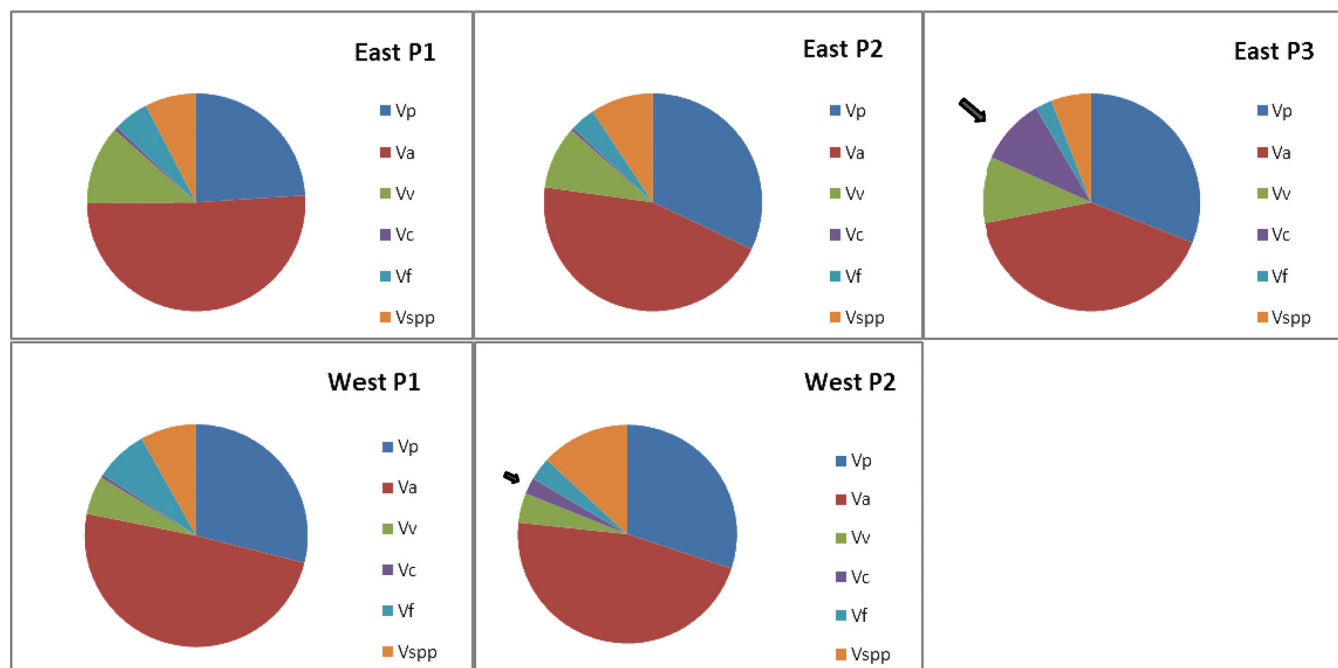
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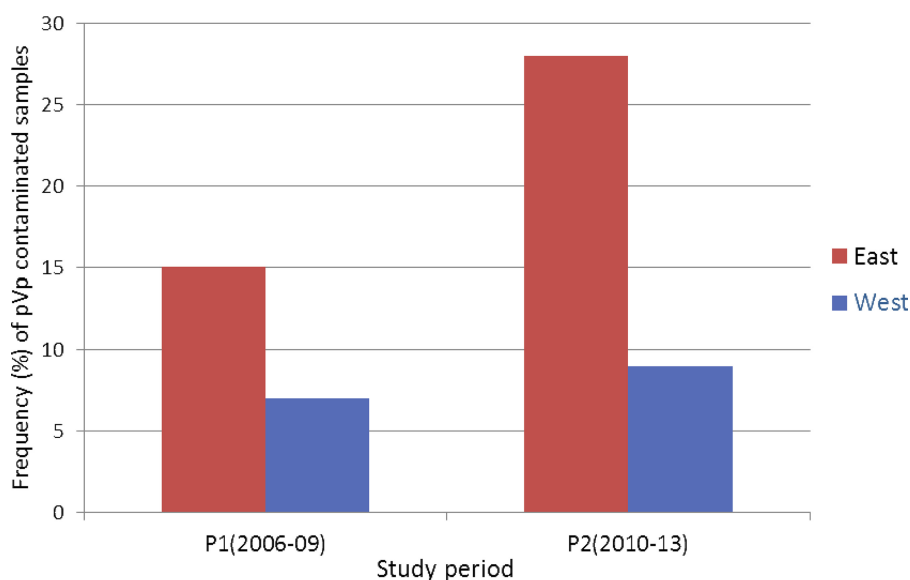


**FIG 1** Normalized (percent) proportions of the individual *Vibrio* species detected during the study period, divided into segments/periods (P) to observe the trend and dynamics. Vp, *V. parahaemolyticus*; Va, *V. alginolyticus*; Vv, *V. vulnificus*; Vc, *V. cholerae*; Vf, *V. fluvialis*; Vspp, *Vibrio* species; P1, 2006 to 2009; P2, 2010 to 2013; P3, 2014 to 2016. The arrows point to the significant increases in Vc prevalence.

## RESULTS AND DISCUSSION

During the 11-year period (2006 to 2016), five identified *Vibrio* spp., comprising halophilic (*V. parahaemolyticus*, *V. vulnificus*, *V. fluvialis*, and *V. alginolyticus*) as well as nonhalophilic *V. cholerae* strains, were isolated with various frequencies of detection (Fd). In addition, unidentified *Vibrio* spp. of marine origin were detected in large numbers and were included in the analysis (Fig. 1). As seen in Fig. 1, the Fd for *V. parahaemolyticus* increased from 41% (2006 to 2009, P1) to 69% (2010 to 2013, P2) and 63% (2014 to 2016, P3) for the east coast molluscan samples, while the values were 57% (P1) and 62% (P2) for the west coast samples during the defined periods. The values for *V. alginolyticus* remained more or less stable but high (83% to 97%). The tropical species *V. cholerae* emerged with an increased Fd in the later periods. On the east coast, the Fd for *V. cholerae* jumped to 20% (P3) from 1% (P1 and P2), while on the west coast, the value increased from 1% (P1) to 5% (P2). The Fd values for *V. vulnificus* were more or less stable on either coast, with 20% for the east (P1, P2, and P3), and 11% (P1) to 9% (P2) for the west. Unidentified *Vibrio* spp. were detected in the range of 13% (P1, east) to 27% (P2, west). Data for the west coast molluscs during P3 are not included, as samplings were interrupted, and the few available data will have to be repeated and merged with ongoing data. This study revealed the presence of a trend in the composition of the *Vibrio* community in Canadian coastal waters, which may be driven by climate change. The observed composition in interspecies diversity of the genus *Vibrio* with increasing abundance of *V. cholerae* strains, which are endemic in the tropical regions of the world, supports the notion that *V. cholerae* strains are emerging in the Canadian waters. Follow-up studies are required to investigate the influence of environmental factors on the *Vibrio* community composition associated with the specific harvest sites. Historically, cholera is known to have reappeared in areas from where it had been absent for many years, and it has also emerged in newer communities, resulting in an expanded area of endemicity (4).

Coinciding with the increasing emergence of *V. cholerae* isolates, there was also an increase in the number of molluscan *V. parahaemolyticus* isolates testing positive for virulence markers by PCR (Fig. 2). Coincidentally, the rise in frequency of potentially pathogenic *V. parahaemolyticus* strains was significant on the east coast (14.6% in P1 to



**FIG 2** Frequency of harvested molluscan shellfish samples testing positive for potentially pathogenic *V. parahaemolyticus* (pVp). The data were averaged over successive 4-year periods and normalized (percent), as shown, to observe the trend.

27.9% in P2), as opposed to the trend on the west coast (6.6% in P1 to 9.4% in P2). During the same study period, *V. parahaemolyticus* isolates increasingly tested positive for the pathogenic hemolysin genes (*tdh* and *trh*) associated with gastroenteritis in human beings in Canada. Seasonality is attributed to *V. parahaemolyticus* infections, as this organism causes most of the illnesses in the warmer months of the year in temperate regions (3, 14). The dynamics of *V. parahaemolyticus* infection and the diversity of the causative strains testify to the existence of many variants of this species as a result of its genome plasticity and adaptability to climate changes (1, 3, 4). Hence, the impact of the warming trend on the proliferation and abundance of disease-causing hemolysin genes in molluscan *V. parahaemolyticus* strains indicates a correlation which requires further investigation.

Both findings indicate that pursuing and studying the Canadian *Vibrio* community over a long period of time will be useful for ecosystem assessment. This scientific information may help develop strategies to reduce the occurrence of illnesses or outbreaks caused by *Vibrio* spp. in seafood and to assess the adverse effects of climate change in Canadian estuaries.

## MATERIALS AND METHODS

Bivalve molluscs (clams, mussels, and oysters), which are filter feeders of planktons and marine pollutants, were sampled from the coastal waters of British Columbia (Canadian Pacific coast) and the Gaspé Peninsula in Québec, as well as the Digby and Yarmouth counties in Nova Scotia (Canadian Atlantic coast), for the presence of *Vibrio* species, particularly *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*, *V. fluvialis*, and *V. alginolyticus*, from May to October of each year from 2006 to 2016. Biochemical and molecular assays, including PCR, targeting the virulent hemolysin genes of *V. parahaemolyticus* were used to identify and characterize the isolates (15, 16). The study period was divided into three parts, P1 (2006 to 2009), P2 (2010 to 2013), and P3 (2014 to 2016), spanning 3 to 4 years and normalized (percent) per year to express the percent prevalence (frequency of detection [Fd]) of each species. Data from the east (Atlantic) and the west (Pacific) coasts of Canada were analyzed separately.

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